Journal of Forestry Research (2014) 25(3): 695–700
DOI 10.1007/s11676-014-0509-3

ORIGINAL PAPER

Scientific analysis of indigenous techniques for guggal (*Commiphora wightii*) tapping in India

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Received: 2012-09-29; Accepted: 2013-01-07

Abstract: Guggal, a threatened species that is endemic to western India, is tapped to extract medicinally important oleo–gum–resin (guggul). However, the plant dies after gum exudation. The indigenous tapping techniques used by local people were examined in Gujarat, India to discover the scientific basis behind these techniques: selection of gum inducer, season of tapping, and plant parts to be tapped. First, the presence of *Xanthomonas axonopodis* pv. *commiphorae* (*Xac*) in the gum suspension used for tapping was established. This bacterium induces gum oozing from the tapped plants and later, causes them to die off. The population of *Xac* in gum was found to decrease with the age of the gum. With that, fresh gum increased the tapping success. Second, local people preferred tapping during the warm season, which we validated by determining that *Xac* growth was best at 30 °C. Tapping during September (mean temperature 25.7–30.0 °C) clearly favoured growth of the pathogen and yielded maximum guggul. Multiple tapping on a mature tree ensured maximum gum extraction before its death. Finally, application of indigenous technology under natural plant stands by the local people ensured availability of this important raw drug for consumption. Our study established that the age–old traditional methods have a strong scientific basis. However, it is imperative to formulate strategies based on contemporary scientific understanding to protect this natural resource before it becomes extinct.

Keywords: *Burseraceae,* gummosis, medicinal plant, participatory forest management, xerophytes

Project funding: This study was financially supported by the National Medicinal Plants Board, New Delhi.

The online version is available at http://www.springerlink.com

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Corresponding editor: Chai Ruihai

Introduction

Guggal [*Commiphora wightii* (Arn.) Bhand., family Burseraceae] is a small tree or shrub found naturally growing on the rocky, arid, semi–arid, and hilly regions of the western Indian states, Gujarat and Rajasthan. Diverse species of *Commiphora* are distributed in different parts of Asia and Africa and their oleo–gum–resins are used for various purposes (Mcdowell et al. 1988).

Guggal is tapped by the local people for extraction of medicinally important oleo–gum–resin (guggul). *Ayurveda* recommends this drug for treating a condition "coating and obstruction of channel," resembling the description of atherosclerosis (Satyavati 1966). The modern therapeutic uses of guggul are targeted against rheumatoid arthritis, nervous system diseases, and hypercholesterolemia (Srivastava et al. 1984). Recent research studies claim that guggul is effective against cardiovascular diseases (Deng 2007) and cancer (Xiao and Singh 2008). It is also used for preparation of incense.

Annual guggul production in India was about 51 metric tons during 1963–64 (Atal et al. 1975) which has diminished considerably with the dwindling plant stand. Faulty tapping technique is identified as one of the major reasons for the decline in natural guggal population (Soni 2008). The Rajasthan government has banned guggal tapping to tackle this problem. However, it is still unregulated in Gujarat at the non–forest areas, usually at the outer fringe of main forests. Often, boundary limits are not adhered to and clandestine tapping is done in the core areas as well. Collected guggul is marketed through the Gujarat State Forest Development Corporation Ltd. (GSFDCL). Mainly three communities – Sama, Sumra, and Koli practice tapping using their own traditional techniques.

Many tree species, such as *Acacia* spp. (family Mimosaceae) and *Pinus* spp. (Family Pinaceae), are commercially tapped for extraction of resin and gum. However, these tree populations are not adversely affected, unlike guggal trees which die after tapping. Even salai guggal (*Boswellia serrata* Roxb.), a member of

same family that of guggal, will not die following tapping and gum oozing. The natural population of guggal has declined so much that the plant is placed under the IUCN Red List of Threatened Species (www.iucnredlist.org).

Clearly, ad hoc tapping by locals resulting in death of the plant needs critical examination. Our aim here is to provide important clues to develop better management practices to keep guggal from becoming extinct.

With this view, a thorough survey of two major guggul-producing districts of Gujarat, Kachchh, and Bhuj (Lakhpat, Trombo, Nakhatrana, Bhuj talukas) and Jamnagar (Dwarka and Bet–dwarka) was conducted during 2009 to document the indigenous knowledge involved with guggal tapping (Mandal et al. 2011). Local people make multiple bark–deep incisions on the stems of trees and use a suspension or slurry of guggul to flood the injured area. Gum starts oozing after few days from these sites. As a follow–up of this survey, we applied some of the techniques described by the local people to tap guggal plants at the research farm of Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat, India.

Plant death following gum oozing could be due to physiological disturbances or involvement of pathogens in the process. Earlier, we established the association of a pathogenic bacterium *Xanthomonas axonopodis* pv. *commiphorae* (*Xac*) with gumming and death of guggal (Samanta et al. 2012).Consequently, special emphasis was given to determine presence of *Xac* in gum suspension. Objectives of the present study are to determine the scientific bases of indigenous techniques in terms of (1) gum quality used as inducer, (2) season of tapping, and (3) selection of plant and location for tapping.

Materials and methods

Isolation of bacteria

Bacteria associated with plant material were isolated by standard methodology (Samanta et al. 2012). For a gum sample, a suspension was made by mixing 200 mg gum in 5 mL sterile water. Bacteria were isolated from this suspension by serial dilution, using spread plate technique on nutrient agar (NA), and the total bacterial population was estimated as colony-forming units (cfu) g^{-1} gum. Bacterial colonies were picked and streaked again on NA to select the isolated single colonies. Pathogenicity of the bacteria was confirmed by establishment of Koch's postulate.

Estimation of bacterial population in gum and determination of tapping success

Oozed gum was collected 28–30 days after tapping. Bacteria concentration in the gum samples (r=3) were estimated at 0, 7, 15, 30 and 90 days after gum collection by dilution plating. Infectivity of such gum samples was tested by tapping healthy plants (r=3) using the same gum suspension as inducer. Tapping was done by making a semi–circular incision that was immediately covered with a cotton swab soaked in inducer. The swab was wrapped with a Parafilm® strip for 24 h to keep it moist, and both cotton swab and Parafilm were removed thereafter. Plants were observed for 15 days for gum-oozing symptoms.

Tapping success for variable concentrations (10^2, 10^4, and 10^8 cfu mL^{-1}) of *Xac* were determined by inoculating four-month old rooted cuttings in plastic pots. Each treatment was replicated 4 times. The plants were kept under observation up to 15 days for gum-oozing symptoms from inoculated sites. All the experiments were repeated two times, and representative data from one experiment are presented as results from both the experiments were statistically similar.

Determination of effect of incubation temperatures on the growth of *Xac* and tapping success

Optimum temperature for bacterial growth was determined by monitoring the turbidity of the broth culture after 24 h of incubation at 620 nm in a spectrophotometer. Flasks (r=3) containing 100 ml nutrient broth were inoculated with bacteria and incubated at five different temperatures (15, 20, 25, 30 and 35 °C) as stationary culture. The experiment was repeated two times and average data were presented.

The effect of temperature on gum oozing was determined by inoculating six month-old rooted guggal cuttings. Individual cuttings were planted in plastic pots and established plants were shifted into incubators maintained at different temperatures (15, 20, 25, 30 and 35 °C). After 48 h of acclimation at a particular temperature, the plants were tapped following the method described earlier. Control plants were treated similarly except that the cotton was moistened with sterile distilled water. Four plants were used for each treatment and there were three replications for each treatment. Time taken for initiation of gum oozing was noted up to 15 days after inoculation. The experiment was repeated two times and average data from one experiment are presented here.

Tapping under field conditions and guggul yield estimation

Field-grown plants (>5 years old, r=4) were tapped in four different seasons—post winter (February), summer (May), post–rain (September) and winter (December)—with ~10^8 cfu ml^{-1} bacterial suspension. Exuded gum was collected from the tapped positions and yield was recorded.

Different positions (main trunk, M; primary branch, P; secondary branch, S and tertiary branch, T) of mature guggal plants (>7 years old) were tapped to determine gum yields. Each tree was considered as a replication and 10 replications were maintained for each treatment. In another experiment, five different treatments, comprising different combinations of tapping sites were maintained. Individual plants were tapped at T1: 2 positions (P+P); T2: 3 positions (P+S+S); T3: 4 positions (P+P+S+S); T4: 4 positions (M+P+S+S); and T5: 5 positions (M+P+P+S+S). Three replications were maintained for each treatment in this experiment. Two months after inoculation the accumulated gum was collected, weighed, and yield per plant was noted.

Statistical analysis

Data were analysed using the statistical package MSTAT-C version 1.4 (Crop and Soil Science Division, Michigan State University, USA). Pair–wise comparison of means was done using the least significant difference (l.s.d.) procedure (P=0.05).

Results

Association of bacteria with gum oozing

Besides *Xac*, two types of bacterial colonies were isolated from guggal bark or gum samples. These colonies – yellow or whitish yellow in colour – appeared within 24 h of inoculation. *Xac* initiated gum oozing from the site of inoculation within 7–8 days after inoculation. However, control plants (water inoculated) and those inoculated with yellow/whitish yellow cultures did not exhibit such symptoms.

Xac population in gum and tapping success

The *Xac* population in gum samples fell significantly the longer the gum was stored (Fig. 1). The highest concentration of *Xac* (2.4×10^8 cfu g^{-1}) was observed in fresh gum samples. However, their concentration fell gradually over time and the lowest was found 90 days after collection. Interestingly, a conspicuous reverse trend was observed in the population of saprophytic bacteria (data not shown). Suspensions made from such gums produced differential tapping success (Fig. 1). Fresh and 7 day-old gum when used for tapping induced oozing in all the tapped plants thereby ensuring 100% tapping success. Gradual decrease in tapping success was observed with aged gum samples and the 90 days old gum sample failed to induce gum oozing in any tapped plants.

Differential tapping success was also observed in inoculation with variable concentrations of pathogenic bacterium. At the end of the experiment (15 days post inoculation), tapping success was the highest (100%) with 10^8 cfu ml^{-1} inoculum. Tapping success gradually decreased with the decrease in bacterial concentration in inoculum. Tapping with 10^4 cfu ml^{-1} induced gum-oozing symptoms in 40% of the tapped plants and further dilution of bacterial suspension (10^2 cfu ml^{-1}) failed to produce any gum-oozing symptoms. All the plants those showed gum-oozing symptoms died gradually within 35 days of tapping.

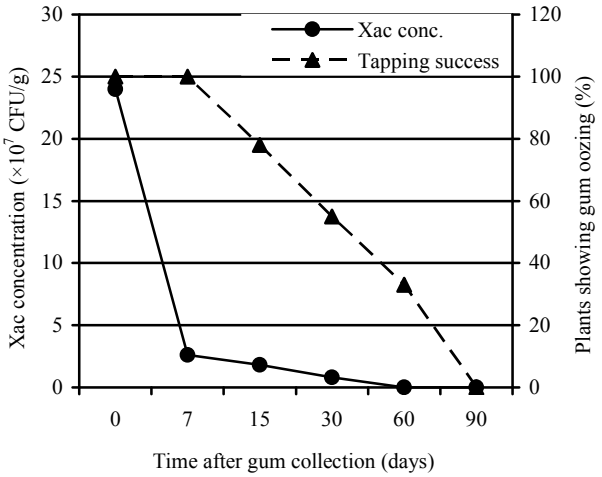

Fig. 1: Bacterium (*X. axonopodis* pv. *commiphorae*) population in guggul at different time after its collection and its effectiveness for tapping.

Effects of temperature on bacterial growth and tapping success

Xac clearly showed preference toward higher temperatures for growth (Fig. 2). In broth culture, the pathogen produced optimum growth at 30 °C followed by 35 °C. The minimum growth was recorded at 15 °C.

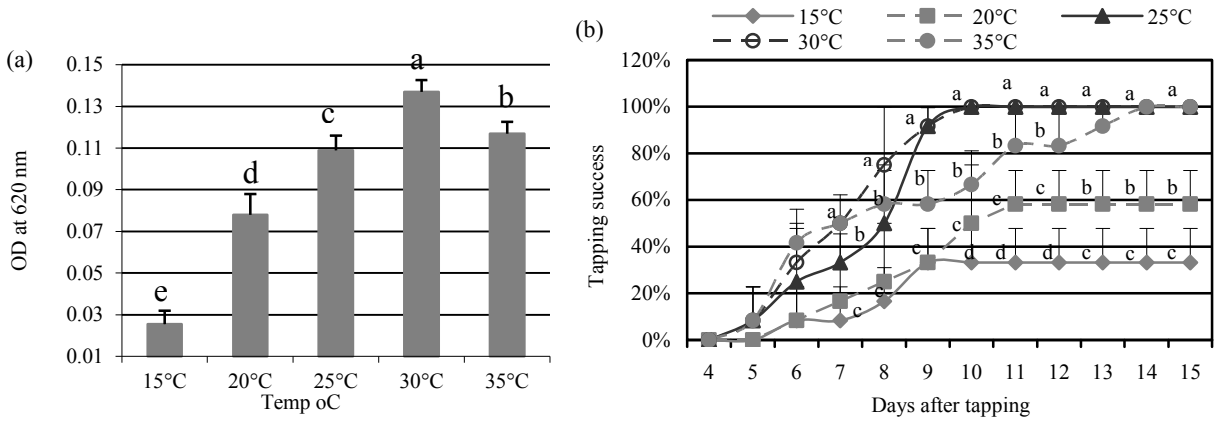

Fig. 2: Effect of different temperatures on growth of *X. axonopodis* pv. *commiphorae* (a) and tapping success (b). Data points from same observation time marked with same letter do not differ significantly (p=5%).

In host-inoculation studies also, higher temperatures induced early gum oozing (Fig. 2). Gum oozing started as early as 4 days after inoculation when plants were incubated between 25 and 35 °C. However, incubation temperature of 30 °C proved to be the best with early initiation of gum oozing and the highest tapping success. Initiation of gum oozing was observed after 6 days of

tapping when the plants were incubated at 15 and 20 °C. Tapping success varied considerably with variations in temperature. Within 10 days of tapping, all of the plants incubated at 25 and 30 °C showed gum-oozing symptoms, whereas 67% of the plants incubated at 35 °C exhibited positive symptoms. By the end of the experiment, all the tapped plants incubated at higher temperatures (25–35 °C) produced gum-oozing symptoms. Such plants ultimately died after gumming. However, tapping success gradually decreased with lower temperatures – 58% at 20 °C and only 25% at 15 °C.

Influence of season and position of tapping on gum yield

Tapping seasons also significantly influenced guggul yield (Fig. 3). Plants tapped in the month of September produced the highest gum (49.4±14.4 g plant^{-1}), while it was the lowest in plants tapped during May (3.1±0.6 g plant^{-1}). December and February tapping produced similar gum yields. Mean temperature during September varied between 25.7 and 30.0 °C while, during May, it was relatively high (mean temperature 32.9–35.1 °C).

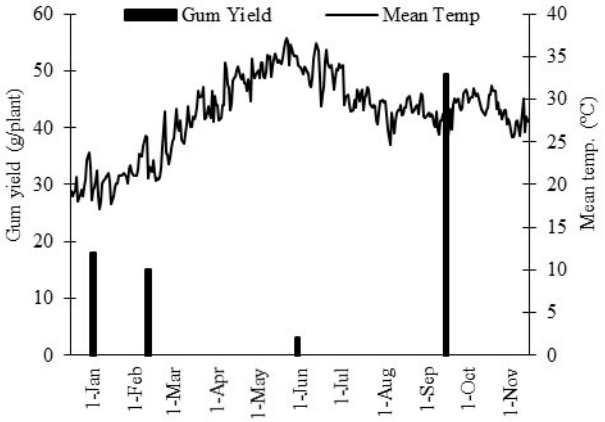

Fig. 3: Mean temperatures and guggul yields at different period of the year.

From a single site, the highest gum yield (81.1±15.1 g plant^{-1}) was obtained from main branch, while tapping on twig produced the lowest gum (2.7±0.7 g plant^{-1}). Tapping at primary and secondary branches resulted in intermediate gum yields (22.1±3.1 and 9.9±1.5 g plant^{-1}, respectively). It was observed that gum yield was proportional to thickness of the tapped stem (R^2=0.97, Fig. 4).

Gum yield per plant was maximized by tapping at multiple sites. Plants tapped at five positions produced the highest gum (175.0±9.0 g plant^{-1}), while it was the lowest when tapped at two primary branches (71.0±6.6 g plant^{-1}). Tapping at three sites (one primary branch and two secondary branches) produced 87.7±4.5 g plant^{-1} gum, while inoculations at four sites (two primary and two secondary branches or, main trunk + one secondary branch + two primary branches) did not differ in gum yield (128.7±15.3 and 134.7±7.4 g plant^{-1}, respectively). There was no difference in plant survival after gum exudation as all such plants died gradually.

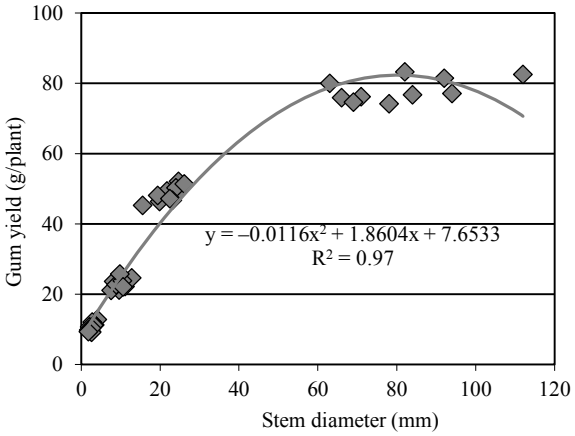

Fig. 4: Scatter plot diagram showing relationships between guggul yield and stem diameter of guggal plant.

Discussion

Death of guggal plants after tapping is an intriguing problem, more so, because of the species' dwindling population under natural habitat. Hence, the present study was undertaken to know if scientific understanding of the traditional practices can lead to better management of tapping to save this endangered species. Indigenous technical knowledge and related practices, developed outside the formal education enable communities to sustain in a particular environment (Warren 1991). Since these are time tested, many feel, these need no validation (Brook and McLachlan 2005; Michell 2005) while others opine that these need to be validated by contemporary scientific knowledge for their holistic application in real life problem solving (Huntington 2000; Dickison 2009).

Xac was earlier established as a causal agent for gummosis of guggal (Samanta et al. 2012). The present study established that the same bacterium was present in gum suspension used for tapping. Plant pathogenic bacteria need an opening to enter into host tissues for successful pathogenesis (Agrios 2005). In the present case, incisions made on the stem permit entry for the pathogen. The presence of pathogen in gum makes it a suitable choice as inoculum to initiate infection. Over the years, local people applied this inoculation technique to infect the plant and extracted oleo–gum–resin from this species.

However, this tapping practice leads to infection of guggal plants by the pathogen culminating in their death. We observed some people performing a ritual, "*pani chadana*," just before tapping (Mandal et al. 2011). This involves flaming the tool used for making incisions. With knowledge of the association of pathogen in gum oozing, it is now easy to understand the necessity of sterilising the inoculating tool. However, without being aware about involvement of the pathogen, the locals' practicing *pani chadana* arouses an element of astonishment.

Traditional guggul extractors use a suspension made from fresh gum to potentiate gum oozing to increase success rate

(Mandal et al. 2011). In the present study, we have seen that guggul contains the pathogenic bacterium responsible for gum oozing. However, the bacterium population decreases with time and so does tapping success. Also, it was observed that decreasing pathogen concentration resulted in the reduced chance of successful gum oozing (Fig. 1). This proves that selection of gum to initiate tapping is crucial for success in gum extraction. Non–practitioners usually fault at this step and achieve low tapping success. This in turn resulted in the origin of several myths associated with supernatural powers of traditional gum extractors (Mandal et al. 2011).

Apart from a susceptible host and a virulent pathogen, environment plays a major role in the infection process leading to disease development (Agrios 2005). Pathogens have temperature optima for growth and multiplication and similarly hosts perform best within a certain temperature range. Disease development is influenced by such factors and varies with the host–pathogen interactions. The most rapid disease development occurs when prevailing temperature supports growth of the pathogen but is away from the comfort zone for the host (Agrios 2005). In the present study, the shortest time to develop symptoms was at 30 °C. Under *in vitro* conditions, the pathogen exhibited best growth rate at 30 °C, corroborating the inoculation study. Guggal is distributed naturally in hot and arid regions. Preference for high temperature by the pathogen for growth and infection supports co–evolution of the pathogen with the host (Mkandawire et al. 2004). Traditionally, guggal tapping and guggul collection are practiced during November–April (Atal et al 1975; Mandal et al. 2011).

During our previous survey, local people informed us that very cold (December–January) and extremely hot (March onwards) weather were not favourable for tapping (Mandal et al. 2011). Based on our findings, such failures are likely due to mismatch between the temperature requirement for pathogen's growth and prevailing environmental conditions. Tapping experiments conducted during different seasons also indicate that temperature was an important determinant of tapping success and guggul yield. Mean temperature during September was closest to the optimum requirement for pathogen's growth and therefore, it resulted in the highest guggul yield.

Local people practice tapping for their livelihood. Hence, maximizing guggul yield from a plant has been their top interest. Multiple tapping on a plant is known to help increase the gum extracted and such excess tapping is believed to result in plant death (Soni 2008). However, this notion is disproved by our study; all plants from which guggul was obtained by tapping, irrespective of the number of sites it was tapped, faced slow death. Therefore, maximizing guggul yield through multiple tapping is a better proposition.

Tapping at multiple sites of a same tree is practiced in other species such as, *Boswellia papayrifera* to maximise yield (Mengistu et al. 2012). Local people avoid smaller trees for tapping on account of their low yield (Mandal et al. 2011). We also established that guggul yield was proportional to stem thickness, thereby validating people's practice of selecting only mature trees for tapping because of their higher yield potential before eventual death (Fig. 4).

On the basis of our results, we conclude that indigenous knowledge of the three aspects of guggal-gum tapping discussed here has strong scientific basis: (1) fresh gum suspension is used by the locals as it is the best source of pathogen; (2) choice of warm season for tapping helps in optimum growth of *Xac*; and (3) multiple tapping of mature trees helps in better gum extraction. All these are standardized by the indigenous people to fulfil their need—to maximize guggul yield.

Association of bacterium with gum extraction may not have been known to the people engaged in tapping but, with years of observation, experimentation, and practice they have improved the technique to its best. This study also verifies that post–tapping death of guggal trees is due to infection by the pathogenic *Xac* present in the gum suspension. For this reason, a balance needs to be maintained between medicinally important guggul extraction and efforts to save this threatened species from becoming extinct. Increasing the efficacy of guggul extraction is one possible means to mitigate over exploitation. Cultivation methods, such as fertilization and irrigation, can increase plant growth and, in turn, guggul yield. Mengistu et al. (2012) reported that frankincense yield from *B. papyrifera* was not correlated with photosynthetic carbon gain. However, in the case of guggal, soil moisture of 25–30% was found to increase the photosynthetic rate and plant growth, thereby enhancing guggul yield (Samanta et al. 2012a).

We suggest three steps to save guggal in its natural habitat: (1) impose a blanket ban on guggal tapping in the forest and adjoining areas; (2) build commercial guggal plantations for gum extraction; and (3) develop partially resistant lines of guggal against the pathogenic *X. Axonopodis* that produce gum but still can survive the infection.

Acknowledgements

The authors are thankful to the Director, DMAPR, Anand for providing facilities to conduct this study. Financial help provided by the National Medicinal Plants Board, New Delhi is gratefully acknowledged. We specially thank the staff members of GSFDCL and the local people of Kachchh for their cooperation during the field work.

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